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SELECTIVE FORMYLATION OF AMINO GROUPS UNDER NEUTRAL CONDITIONS

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One of us reported recently[1] the synthesis of isopropenyl formate and mentioned its high formylating capacity, especially when neutral conditions are needed. This characteristic should make it an interesting reagent.

In this paper we demonstrate the reactivity and selectivity of isopropenyl formate and its usefulness in blocking or protection of amino functions.

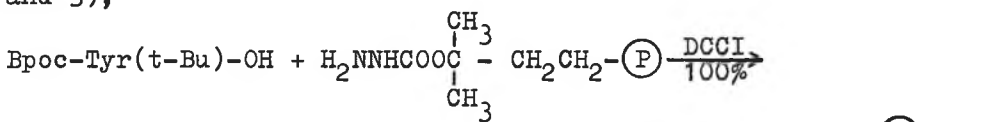
In several preliminary experiments formylation of amines by isopropenyl formate,

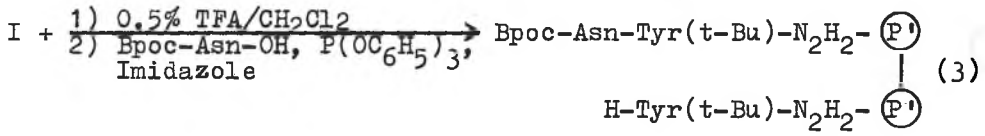
$$RR'NH + HCO\text{-}OC(CH_3)=CH_2 \longrightarrow RR'N\text{-}CHO + CH_3COCH_3 \qquad (1)$$

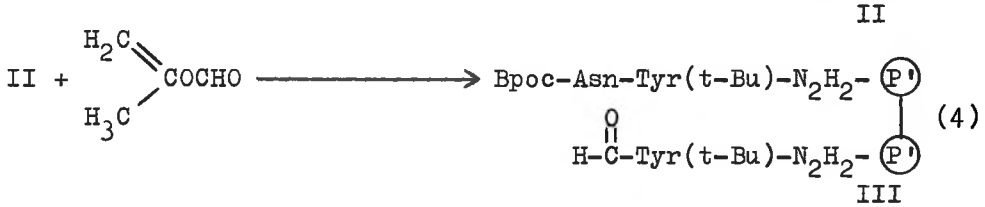
was followed by NMR. With propyl-, benzyl- and diethylamine the strongly exothermic reaction was complete within five minutes. Aniline was formylated in three hours at room temperature. With N-methylaniline and p-chloroaniline a reaction time of 12 hours appeared to be necessary. Conversion of methanol or phenol could not be observed under these conditions; only after ten days did methanol give 30% of methyl formate, phenol less than 2% of phenyl formate.

As these results indicated that the reagent could be very useful in peptide synthesis, we tried selective amino formylation of an amino acid ester. In dichloromethane N-formylation of the methyl ester of tyrosine was performed within 15 minutes while the hydroxy group remained unchanged. In DMF a similar result was obtained within 60 minutes.

In the solid-phase synthesis of part of the A-chain of ovine insuline on a tert-alkoxycarbonyl hydrazide resin[2] blocking of unreacted free amino groups was felt necessary after an incomplete coupling step. Acidic reagents like the formic acid/acetic anhydride mixture[3] could not be used because of the presence of acid-labile Bpoc-groups. A typical example is represented in the following scheme (reactions 2 and 3),

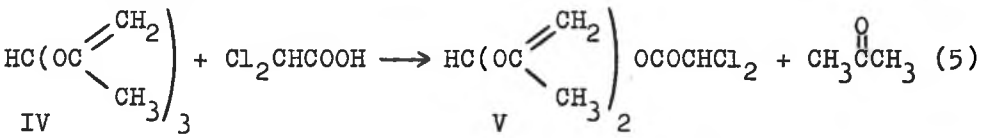


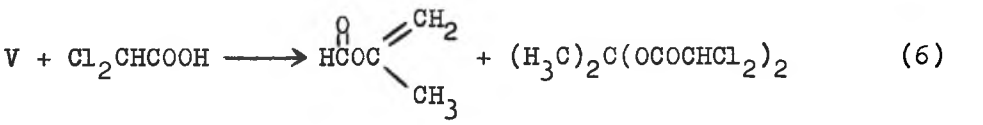




Free amino groups left after the coupling step (3) were formylated by isopropenyl formate (4). By determination of free and blocked amino groups after the reaction it could be shown that almost all amino functions had been protected. Isopropenyl formate could also be used for blocking of the hydrazide function of the resin, if coupling of the first amino acid with the resin was incomplete.

Isopropenyl formate was synthesized[4,1] according to the following scheme:





$$V + Cl_2CHCOOH \longrightarrow HCOC(=CH_2)CH_3 + (H_3C)_2C(OCOCHCl_2)_2 \qquad (6)$$

To obtain pure isopropenyl formate it is neceassary to isolate the intermediate V because it is difficult to separate isopropenyl formate from acetone (method a). With most formylations the presence of acetone does not interfere, and the reagent can be prepared in a simple one-step procedure (method b).

Experimental

For all new compounds correct elemental analysis were obtained.

1. Isopropenylformate

method a: Isopropenyl formate was prepared by adding dropwise one equivalent of dichloroacetic acid to V under diminished pressure (20 mm Hg) while the temperature in the reaction flask was kept at about 40°C. The product evaporated from the reaction mixture and was collected in a Dry Ice cooled vessel. Yield 80%, b.p.80-81°C, n_D^{20}=1.3990. Immediate isolation of the compound is essential because it is acid-labile.

method b: Upon addition of two equivalents of dichloroacetic acid to IV under the conditions described above, a 1:1 mixture of isopropenyl formate and acetone was isolated in a yield of 90%.

2. N-Formyl-L-tyrosine methyl ester

195 mg. (1 mmole) of L-tyrosine methyl ester was suspended in 1 ml of CH_2Cl_2. After the addition of 0.2 ml of a 50% solution of isopropenyl formate in acetone (10% excess) a clear solution was obtained. After 15 minutes thin layer chromatography showed the absence of free amino groups. After one hour a precipitate was formed which after the addition of ether was filtered and dried. The yield of N-formyl-L-tyrosine methyl ester was 212 mg. (95%), m.p.144-145°C, $[\alpha]_D^{22}$= +38.4° (c=1, MeOH).

3. Formylation of Bpoc-Asn-Tyr(t-Bu)-NHNH-resin

Bpoc-Tyr(t-Bu)-NHNH-resin was prepared in the usual way from Bpoc-Tyr(t-Bu)-OH and the Merrifield tert-alkoxycarbonylhydrazide resin (2). It could be shown that this coupling was quantitative. Determination of the tyrosine content of the resin revealed 0.41 mmole of tyrosine/g. resin. The coupling of the second amino acid derivative, Bpoc-Asn-OH, was performed according to the method of Mitin[5] with triphenyl phosphite and imidazole. Quantitative determination by UV of 2-(p-biphenyl)-propene, liberated in a sample of the mixture, showed that only 82% of asparagine had been incorporated. Electrophoresis (pH=4.6, 400 V, 1 hour) showed two spots, one from H-Asn-Tyr-N_2H_3 and a minor one from H-Tyr-N_2H_3. The resin was treated for 3 hours with a tenfold excess of isopropenyl formate in CH_2Cl_2. Free amino group determination with 2-hydroxy-1-naphthaldehyde[6] revealed only 0.015 mmole free amine/g. resin. Cleavage of the peptide with 50% TFA/CH_2Cl_2 followed by electrophoresis showed two spots, one for H-Asn-Tyr-N_2H_3, and a minor one for N-Form-Tyr-N_2H_3. The latter compound was identified by means of an authentic sample, synthesized from N-Form-Tyr-OCH_3 and hydrazine hydrate, m.p.218-220°C, $[\alpha]_D^{22}$= +16.7° (c=1, DMF).

Acknowledgment

We thank Dr. J.W. Scheeren and Prof. R.J.F. Nivard for discussing the work.

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SYNTHESIS AND PROPERTIES OF L-α-AMINO-γ-NITROGUANIDINOBUTYRIC ACID*

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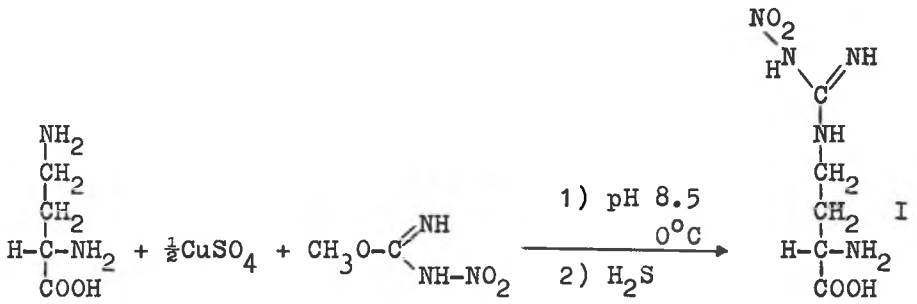
We wish to report the synthesis of L-α-amino-γ-nitroguanidinobutyric acid, a hitherto unknown amino acid derivative. This derivative can be the base for preparing peptides (for instance hormones) containing L-α-amino-γ-guanidinobutyric acid, the lower homologue of L-arginine. The higher homologue, L-homoarginine, has already been built in in peptides through its nitroderivative, as was the case with angiotensin II analogues[1] and Har-Bradykinin[2].

L-Nitrohomoarginine and L-nitroarginine are prepared by treatment of L-lysine or L-ornithine in alkaline solution (pH 11) with $CuCO_3$, and then at about 0° with 2-methyl-1-nitroisourea under stirring[3]. We prefer to use $CuSO_4$ instead of the carbonate. An attempt to synthesize L-α-amino-γ-nitroguanidinobutyric acid from L-α,γ-diaminobutyric acid[4] by a similar procedure was not successful. At pH 8.5 however, we could isolate the copper complex of L-α-amino-γ-nitroguanidinobutyric acid in 56% yield.

* Suggested abbreviation for this lower homologue of L-nitroarginine: H-Agb(NO_2)-OH.



In a typical experiment 7.73 g (=50 mmol) of L-α,γ-diaminobutyric acid monohydrochloride were placed in a reaction flask and dissolved in about 60 ml of water. To the solution $CuSO_4$ (4.00 g = 25 mmol) was added under magnetically stirring. The flask was placed in an ice bath, the pH of the solution was adjusted to 8.5 and kept constant during the following reaction with the aid of an autotitrator (Radiometer, Kopenhagen) and 4N NaOH. Subsequently, 6.66 g (=56 mmol) of 2-methyl-1-nitroisourea were added in the course of a few hours.
After about four hours no more NaOH solution was consumed. The pH was adjusted to 7 with acetic acid and after cooling the light-blue L-α-amino-γ-nitroguanidinobutyric acid-copper complex was filtered and washed with water, aceton and ether to yield 6.63 g = 56.2% of a pure product.

The low yield might be explained by the presence of the γ-amino group which competes with the carboxyl group in complexing the copper ions[5], leading to a soluble complex.

The amino acid was liberated by bubbling of H_2S through a solution of the complex in dilute hydrochloric acid, water and methanol. The CuS was filtered off and the filtrate concentrated. After addition of some methanol, the pH of the solution was adjusted to about 6. After cooling 4.02 g = 62% of the white, crystalline L-α-amino-γ-nitroguanidinobutyric acid could be isolated. A sample was recrystallized from water; m.p. 206.5-208.5°C (dec.160-175°C), $[\alpha]_D^{23}$ = +35.6° ± 1° (c=1, 1N HCl); +35.2° ± 1° (c=1, 2N HCl); $\mathcal{E}$ = 15,810 (at λ_{max}=268 nm in D.M.F./0.2 N HCl, v/v 1:1[6]), $\mathcal{E}$ = 1,810 (at λ_{max}=265 nm in T.F.A.).

Found: C 26.83%; H 5.84%; N 31.34%.
Calcd. for $C_5H_{11}N_5O_4.H_2O$: C 26.91%; H 5.87%; N 31.38%.

For use in peptide synthesis either the α-amino or the carboxyl group of I has to be protected. To this aim we tried to prepare the t.-butyloxycarbonyl derivative according to Schnabel[7]. However, on thin layer chromatograms the reaction mixture showed six spots. This phenomenon is probably due to the instability of I in alkaline medium.

From the literature[8] it is known that the stability of L-nitroarginine at high pH values is also restricted; in a nearly 2N NaOH solution at room temperature less than 2% of 2-nitrimino-4-carboxy-1,3-diazacycloheptane is formed; but after treatment with a 250 fold excess of Na_2CO_3 on a steam bath during one and a half hour, 34% of the ring product has been isolated.

The more pronounced sensitivity towards bases of L-α-amino-γ-nitroguanidinobutyric acid was shown in the following experiments: when a sample of I was treated with 1 N NaOH at room temperature, the resulting solution was ninhydrin negative within one minute and did not reveal UV absorption at 268 nm (typical for the nitro group). However, on dissolution of I in 5% Na_2CO_3 the solution became ninhydrin negative, but did still show the absorption of the nitro group. The same was true for a solution of I in 5% $NaHCO_3$ (pH 8). Obviously, deprotonation of the α-amino group of the zwitter-ionic form of L-α-amino-γ-nitroguanidinobutyric acid triggers a reaction and (as evolution of ammonia was observed) ring-closure to 2-nitrimino-4-carboxy-1,3-diazacyclohexane may be well envisaged.

The preparation of methyl L-α-amino-γ-nitroguanidinobutyrate hydrochloride with thionylchloride and methanol succeeded without difficulties. The yield was 97%, m.p. 91-93°C,

$[\alpha]_D^{26}$ = +18.1° ± 1° (c=1, MeOH p.a.), $\mathcal{E}$ = 14,380 (at 268 nm in D.M.F./0.2 N HCl, v/v 1:1) and $\mathcal{E}$ = 1,790 (at 262 nm in T.F.A.).

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